

## Examples

### General methods

5 Fermentation broth from *Propionibacterium freudenreichii* CBS 929.97 was obtained as described in International Patent Application WO00/37699.

The fermentation broth was concentrated by means of ultrafiltration (on polysulfon MW cut off 5-10 kD, Koch HFK 151 VSV) or microfiltration (on Membralox ceramic 0.1µm) up to a biomass concentration of 100-150 g/l.

10 After ultrafiltration or microfiltration, the propionic acid in the biomass had a concentration of about 25-30 g/l. To reduce the concentration of propionic and acetic acid in the biomass, the biomass concentrate was diafiltered with water. This diafiltration was performed by an in-line addition of water to the concentrate at the same rate as the permeate flow. The diafiltration was stopped at a propionic acid concentration lower than 5 g/l. At this purpose a ratio (v/v) water : concentrate of 3-4 : 1 was applied.

15 After diafiltration the concentrated biomass was pasteurised during 1 minute at a temperature of 90-94°C (either by direct steam injection or heating by a plate heat exchanger).

20 The pasteurized biomass was further concentrated by a multistage (vacuum) falling film evaporator with vapor recompression. This type of evaporator is known to those skilled in the art.

The following conditions were applied.

	Biomass feed rate	2000-3000 l/h (corresponding to 300 kg dry matter/h)
25	Pre-heater temperature	92°C
	1st stage temperature	65-70°C
	5th stage temperature	50-55°C
	Temperature of concentrate	45-50°C
30	Biomass concentration	22-26% (1250 kg/h)

The biomass concentrate was spray-dried on a Multi Stage Dryer (NIRO AS, Denmark).

The following set up was used in all the experiments.

The vitamin B12-containing biomass was fed into the drying chamber by a nozzle  
5 with a biomass feed rate of 1250 kg dry matter/h).

Nozzle pressure	190-195 bar
Air inlet temperature (co current)	200-220°C
Air outlet temperature	75-92°C
Air Internal fluid bed temperature	55-60°C
10 Air 1st external fluid bed temperature	30-35°C
Air 2nd external fluid bed temperature	15-20°C
Powder temperature	< 30°C

Fines were returned via a cyclone to the nozzle area.

15 **(Comparative) Example 1**

In this example vitamin B12-containing biomass was spray-dried in absence of solid carrier applying the above-mentioned spray-drying conditions.

**Example 2**

20 In this example 600 kg of vitamin B12-containing spray-dried biomass obtained in Example 1 was mixed in an external powder mixer (batch) with 300 kg of wheat flour and 1 kg of silica (Aerosil 200®).

**Example 3**

25 In this example 120 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  per kg of concentrate was added to the diafiltered biomass concentrate (120 g/l biomass concentration) before evaporation. The mixture was evaporated to a dry matter content of 32% and spray dried as described above.

**Example 4**

30 In this example vitamin B12-containing biomass was spray-dried in presence of wheat flour as a solid carrier applying the above-mentioned spray-drying conditions. The